EFFECTS OF PANTOPRAZOLE ON XENOBIOTIC METABOLIZING ENZYMES IN RAT LIVER MICROSOMES: A COMPARISON WITH OTHER PROTON PUMP INHIBITORS

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ABSTRACT:
The effects of pantoprazole on xenobiotic metabolizing enzymes in rat liver microsomes were examined. Groups of female Sprague-Dawley rats were orally administered pantoprazole and other proton pump inhibitors, omeprazole and lansoprazole, at 5, 50, or 300 mg/kg/day for 7 days, followed by assays to detect changes in the levels of liver microsomal protein, cytochrome P450, cytochrome b5, NADPH cytochrome c reductase, and drug metabolizing enzyme activities. Increases in total cytochrome P450 contents were evident after a 7-day high-dose administration of all the proton pump inhibitors tested, and the increase by treatment with pantoprazole was less than that with lansoprazole. The three proton pump inhibitors increased the enzymatic activities and cytochrome P450 enzyme levels of CYP1A, CYP2B, and CYP3A. CYP1A was less induced with pantoprazole than with omeprazole or lansoprazole. In contrast, CYP2B was more strongly induced with pantoprazole than with other proton pump inhibitors. NADPH cytochrome c reductase was induced with omeprazole and pantoprazole. The present results suggest that enzyme induction differs among these proton pump inhibitors not only quantitatively but also qualitatively.

PAN1, sodium 5-(difluoromethoxy)-2-[(3,4-dimethoxy-2-pyridinyl)methyl] sulfonyl]-1H-benzimidazolide sesquihydrate, is a drug used in the treatment of peptic ulcers (fig. 1). It is a PPI which binds H+K+-ATPase via a disulfide bond, thereby blocking the active transport of H+ into the gastric lumen (1). Metabolic biotransformations of PAN by P450s have been investigated in detail (2). Phase I metabolic reactions of PAN include mainly S- and methyl group oxidations and aromatic hydroxylations, all of which are typical reactions catalyzed by P450. The hydroxylated metabolites then undergo phase II metabolism, i.e. glucuronidation or sulfation, and are then excreted chiefly in urine. PAN is more highly water soluble and well absorbed from the alimentary tract than OM and LAN which are also substituted benzimidazole PPIs (3, 4).

To date there are no reported signs of toxicity in any animal species for PAN, and its oral LD50 values for mice and rats are reportedly greater than 1 g/kg. It has exhibited no clinically significant interactions with theophylline, antipyrine, diclofenac, digoxin, nifedipine, diazepam, warfarin, and phenytoin (5–9). However, OM increases plasma levels of concomitantly administered diazepam, phenytoin, and certain other drugs, and this effect is attributed to inhibition of P450 (10). In contrast, the repeated administration of OM and LAN also results in the induction of CYP1A2 in vivo in man (11–13), and in vitro studies using cultured human hepatocytes suggest strong induction of CYP1A and CYP3A subfamilies by OM and LAN (14, 15). Thus, PPIs are not only substrates for P450 but also undergo integrated interactions with P450, resulting in either inhibition or

1 Abbreviations used are: PAN, pantoprazole; OM, omeprazole; LAN, lansoprazole; MC, 3-methylcholanthrene; PB, phenobarbital; U, untreated; CMC, carboxymethyl cellulose; EROD, ethoxyresorufin O-deethylase; PROD, pentoxyresorufin O-depentylation; POD, phenacetin O-deethylation; T6H, testosterone 6β-hydroxylation; P450, cytochrome P450; b5, cytochrome b5.

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FIG. 1. Chemical structures of PAN, OM, and LAN.
induction. Some reports are discussed about the induction of CYP1A and CYP3A by OM and LAN (14, 15); however, little information is available in the literature concerning the induction of xenobiotic metabolizing enzymes by PAN. The present study examines the effects of daily oral administration of PAN on xenobiotic metabolizing enzymes in female rats in comparison with OM and LAN by quantifying enzyme levels and measuring specific P450 activities.

Materials and Methods

Chemicals. PAN was supplied by Byk Gulden Lomborg Chemische Fabrik GmbH (Konstanz, Germany). OM and LAN were synthesized by Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan). The chemical structures of these compounds are shown in fig. 1. MC, aminopyrine, 7-ethoxycoumarin, formaldehyde, umbelliferone, phenacetin, 4-aminopyrrolidone, ethoxysorufin, pentoxyresorufin and resorufin were purchased from Sigma Chemical Co. (St. Louis, MO). 14C-Testosterone and unlabeled testosterone were purchased from ICN Biochemicals, Inc. (Lisle, IL), and PB and MgCl2 from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). [14C]-Testosterone and unlabeled testosterone were purchased from Dupont/NEN, Inc. (Boston, MA) and Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan), respectively. NADPH, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). All other reagents used were of analytical grade.

Animals and Treatment. Twelve groups of SD female rats (age: 7 weeks, body weight: 150–180 g) were used and assigned to treatment groups consisting of 0.5% CMC solution, MC, PB, OM (5, 50, or 300 mg/kg/day), OM (5, 50, or 300 mg/kg/day), or LC (5, 50, or 300 mg/kg/day). Each MC-treated group received an MC suspension in corn oil at 30 mg/kg ip once a day for 3 days, and the PB-treated group received a solution of PB in physiological saline at 80 mg/kg ip once a day for 7 days. PAN, OM, and LAN, each suspended in 0.5% CMC solution, were administered orally once a day for 7 days. All animals were weighed at each dose administration and immediately before sacrifice. Rats were sacrificed 24 hr after the last dose. Liver microsomes were prepared using standard ultracentrifugation techniques (16).

Determination of Protein, P450, b5 Content, and NADPH Cytchrome c Reductase Activity. Quantitation of total liver microsomal protein was performed using the method of Lowry et al. (17) with bovine serum albumin (fraction V, Sigma Chemical Co.) as a standard. Determination of P450 and b5 levels were performed as described by Omura and Sato (16, 18). NADPH-cytochrome c reductase activity was measured by the method of Phillips and Langdon (19), with one unit defined as the activity required to reduce 1 μmole of cytochrome c in one min at 25°C.

Assay of Drug Metabolizing Enzyme Activities. Aminopyrine N-demethylation assay was assayed by measuring the amounts of formaldehyde levels, as described in Mazel (20). Ethoxycoumarin O-deethylation activity was determined by measuring umbelliferone formed according to the fluorometric method of Greenlee and Poland (21). EROD and PROD activities were measured by the methods of Phillips and Facts (22) and Lube et al. (23). The fluorometric determination of the increase in resorufin was performed using a Hitachi Model 650–60 spectrophotometer. POD activity was determined by a slight modification of the HPLC method of Kwokward et al. (24), by measuring 4-aminopyrrolidone resulting from deethylation of phenacetin. The HPLC system (Shimadzu Co., Kyoto, Japan) included the following: SCL-10A, system controller; SPD-10A, UV detector; LC-10AD, pump; and DGO-4A, degasser. The column used was Inertsl ODS (GL Science, Tokyo, Japan). The wavelength detector was set to 243 nm and the flow rate was maintained at 1.0 ml/min; using MeOH/H2O as the mobile phase controlled by a gradient program (0–8 min, 30/70; 8–13 min, 55/45; 13–18 min, 30/70). T6H activity was determined by a slight modification of the method described by Waxman (25). The radioactivity in each assay was quantitated by a slight modification of the method described by Waxman (25). The radioactivity in each assay was quantitated by a slight modification of the method described by Waxman (25).
The PAN-treated group exhibited an increased OM, or LAN at the highest dose, 300 mg/kg/day for 7 days were sis. Specific P450 levels in liver from rats orally administered PAN, 2.2-fold increases in this activity, respectively (fig. 5). High doses of OM and LAN also significantly increased this activity (2.9-fold, respectively). PB and MC resulted in 7.3- and 2.5-fold significant increases in the PAN, OM, and LAN at the high-dose treated groups, respectively, as compared with control (fig. 3).

Intergroup comparisons of POD activity demonstrated 3.0-, 3.1-, and 2.5-fold significant increases in the PAN, OM, and LAN at the high-dose treated groups, respectively, as compared with control activity (fig. 4). Similar to EROD activity, the LAN middle-dose group as well as the high-dose group displayed a marked increase in POD activity as compared with control (2.7-fold). Treatment with high dose of PAN resulted in a significant, 2.5-fold, increase in T6H activity, compared with that of the control group. High doses of OM and LAN also significantly increased this activity (2.9- and 1.8-fold, respectively). PB and MC resulted in 7.3- and 2.2-fold increases in this activity, respectively (fig. 5).

Measurement of P450 Enzyme Levels by Western Blot Analysis. Specific P450 levels in liver from rats orally administered PAN, OM, or LAN at the highest dose, 300 mg/kg/day for 7 days were determined (table 2). The PAN-treated group exhibited an increased CYP1A1 content (6.1-fold greater than control). This 6-fold increase, however, was significantly less than that found in the OM- and LAN-treated group (8.2- and 9.3-fold control, respectively). There was a 18.8-fold increase in the content with MC-treated group. CYP1A2 content also increased significantly after treatment with PAN, OM, LAN, and MC (3.6-, 3.9-, 6.3- and 9.9-times the control level, respectively), and the increase in the content in the PAN-treated group was significantly lower than that in the LAN-treated group. A 2.9-fold increase in CYP2B1 occurred in the PAN-treated group, whereas OM and LAN resulted in 2.6- and 2.7-fold increases in this P450, and PB treatment resulted in a 14.9-fold increase. The increase in CYP2B1 by the treatment of PAN was significantly greater than that found by that of OM. Animals treated with PAN exhibited a 5.0-fold increase in CYP2B2 content, which was significantly greater than that seen in the OM- and LAN-treated groups. Microsomal CYP3A2 content increased 1.9-, 2.9-, and 3.0-fold in the PAN-, OM- and LAN-treated groups, respectively, but not to a significant extent.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg/day)</th>
<th>Cytochrome P450 (nmol/mg protein)</th>
<th>Cytochrome b₅ (nmol/mg protein)</th>
<th>NADPH-cytochrome c Reductase (U/mg protein)</th>
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<tbody>
<tr>
<td>Treated</td>
<td>CMC 0.50%</td>
<td>0.643 ± 0.098</td>
<td>0.456 ± 0.014</td>
<td>0.069 ± 0.008</td>
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<tr>
<td>PAN 5</td>
<td>0.641 ± 0.059</td>
<td>0.431 ± 0.013</td>
<td>0.096 ± 0.008</td>
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<tr>
<td>PAN 50</td>
<td>0.700 ± 0.022</td>
<td>0.481 ± 0.032</td>
<td>0.082 ± 0.007</td>
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</tr>
<tr>
<td>PAN 300</td>
<td>0.822 ± 0.003³</td>
<td>0.605 ± 0.019⁴</td>
<td>0.111 ± 0.001⁴</td>
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<tr>
<td>OM 5</td>
<td>0.660 ± 0.054</td>
<td>0.408 ± 0.016</td>
<td>0.097 ± 0.003⁴</td>
<td></td>
</tr>
<tr>
<td>OM 50</td>
<td>0.618 ± 0.160</td>
<td>0.468 ± 0.044</td>
<td>0.111 ± 0.001⁴</td>
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</tr>
<tr>
<td>OM 300</td>
<td>0.966 ± 0.080⁵</td>
<td>0.719 ± 0.021⁵</td>
<td>0.168 ± 0.000⁵</td>
<td></td>
</tr>
<tr>
<td>LAN 5</td>
<td>0.603 ± 0.059</td>
<td>0.376 ± 0.03⁴</td>
<td>0.066 ± 0.006</td>
<td></td>
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<tr>
<td>LAN 50</td>
<td>0.717 ± 0.119</td>
<td>0.532 ± 0.009⁶</td>
<td>0.076 ± 0.007⁶</td>
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</tr>
<tr>
<td>LAN 300</td>
<td>1.06 ± 0.045⁷</td>
<td>0.681 ± 0.072⁶</td>
<td>0.098 ± 0.022³</td>
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<tr>
<td>MC 30</td>
<td>1.11 ± 0.135⁸</td>
<td>0.653 ± 0.055⁹</td>
<td>0.062 ± 0.110³</td>
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<tr>
<td>PB 80</td>
<td>1.68 ± 0.168⁸</td>
<td>0.707 ± 0.066⁸</td>
<td>0.194 ± 0.017³</td>
<td></td>
</tr>
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</table>

Each value represents the mean ± SD for 3 rats. Groups were compared by ANOVA, followed by Fisher’s PLSD method for multiple comparisons. ³ Significant difference from control group, p < 0.05. ⁴ Significant difference from PAN-treated group at the respective dose, p < 0.05. ⁵ Significant difference from PAN-treated group at the respective dose, p < 0.05.
Mean values ± SD for 3 rats.

Female SD rats (7 weeks of age) were administered PAN (300 mg/kg/day), OM (300 mg/kg/day), and LAN (300 mg/kg/day) orally for 7 days. MC (30 mg/kg/day) and PB (80 mg/kg/day) intraperitoneally for 3 days and 7 days, respectively. All were killed 24 hours after the last dose. Each value represents the mean ± SD for 3 rats.

CYP, cytochrome P450; NADPH cyt.c, NADPH cytochrome c reductase.

Groups were compared by ANOVA, followed by Fisher’s PLSD method for multiple comparisons.

* Significant difference from control group, p < 0.05.

† Significant difference from PAN-treated group at the respective dose, p < 0.05.

‡ Significant difference from PAN-treated group, p < 0.05.

§ Significant difference from control group, p < 0.05.
and CYP1A2 were induced with LAN at a lower dose (50 mg/kg/day) where no induction with PAN or OM was observed.

Burke and Mayer (30) demonstrated that in hepatic microsomes prepared from rats treated with PB, PROD activity is induced 100-fold greater than the dealkylation of other n-alkyl substituted resorufin, suggesting that this reaction is specifically mediated by CYP2B1 (28, 31, 32). The results here of PROD activity and Western blot analysis show an increase in the CYP2B subfamily following treatment with three PPIs, especially with PB. Unlike the induction of CYP1A, the order of induction of this enzyme was PAN > OM, LAN. Considering the P450 induction by PPIs, only induction of CYP1A and CYP3A have been investigated in detail, i.e. OM and LAN induce CYP1A and CYP3A in humans (11–15); however, there is no information about induction of CYP2B. This is the first report to evaluate the induction of CYP2B with PPIs. These 3 PPIs are mixed type inducers of CYP1A, CYP2B, and CYP3A. LAN induced CYP2B preferentially rather than CYP1A. In contrast, OM and LAN induced CYP1A rather than CYP2B.

Phenacetin is metabolized chiefly by CYP1A2 (33), whereas POD activity was shown to be increased not only by MC but also by PB treatment at high substrate concentrations (33–35), probably because CYP2B also made contribution to the activity at high substrate concentrations. Here MC and PB treatment resulted in comparable activities when the assays were carried out at the high substrate concentration, thereby supporting previous findings. POD activity increased 2.5- to 3-fold after treatment at the highest doses tested with all of the PPIs and the degree of increase did not appreciably differ among the compounds, while LAN increased it greater than PAN at the middle dose. CYP3A was induced by OM and LAN in human hepatocytes as reported previously (14), and in this study it was induced by all 3 PPIs, also in rats.

It is known that NADPH cytochrome c reductase is induced by PB but is not affected by MC (36, 37). The response of microsomal NADPH cytochrome c reductase contents to treatment with the 3 PPIs tested here varied greatly depending upon the treatment, i.e. treatment with PAN resulted in increase in the contents, similar to those seen with PB treatment, whereas LAN treatment had no effect, similar to the MC-treated rats.

It is not established whether PPIs result in induction of the enzymes at clinical levels. However, therapeutic administration of OM and LAN may induce CYP1A based on findings using caffeine and theophylline as probe substrates (11–13). Furthermore, induction of CYP1A was also demonstrated in patients receiving OM by determining the microsomal cytochrome P450 profile in biopsy specimens from the liver lobules (14, 15). On the other hand, PAN has exhibited no significant induction of CYP1A2 (38), suggesting that CYP1A induction ability of PAN might be lower than those of OM and LAN in humans. Thus, the present findings indicating difference in inducibility of CYP1A between PPIs in rats may provide some usefulness for assessing enzyme induction by these drugs in humans, while their direct relevance to humans is not clear because these induction data in rats were obtained at high doses of PPIs (50 mg/kg/day and 300 mg/kg/day).

In summary, the present results suggest that enzyme induction differs among these PPIs not only quantitatively but also qualitatively, i.e. PAN induced CYP2B preferentially rather than CYP1A in contrast to OM and LAN, which induced CYP1A rather than CYP2B. Further studies are required to clarify the relationships between the liver concentration of PPIs and the induction of the P450 isozymes in human.

References

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